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DEPARTMENT OF THE ARMY
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SEROLOGY AND IMMUNOLOGY AS AIDS IN DIAGNOSIS OF SYPHILIS

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G. Daguet

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The aid of serology and immunology in diagnosis of syphilis should be neither overestimated nor underestimated. It would be just as senseless to base diagnosis of syphilis exclusively on a single serological examination, disregarding clinical examination, as to try to ignore or interpret without sufficient knowledge the information furnished by serology in conformity with the fundamental principles of immunology.

In the presence of cutaneous lesions from a recent syphilis, or of typical manifestations of tardy syphilis, the well-informed clinician will make his diagnosis. However, while the search for antibodies is always made, this is done on the one hand by way of confirmation of the diagnosis, and on the other, as a prelude to long-term surveillance of the evolution of the antibody rate under the influence of treatment. This latter aspect, which is subject to controversy, does not fall within the framework of our study.

Our study comprises the situations in which serology plays an essential part on the diagnostic plane. When examinations are made for the purpose of systematic detection, serology is used to eliminate any suggestion of syphilis or is the only element which reveals a clinically mute latent infection. These examinations are made on sizeable fractions of the population, and nearly 1% of their responses are positive in France.

Once latent syphilis is revealed by serology, the second question which arises is whether this infection is evolutive or not. Is quantitative serology able to throw light on this problem? This

subject has to be discussed with great prudence, for delayed hypersensitivity or allergy induced by the prolonged presence of treponemas in the host counts perhaps more in the pathogeny of tardy lesions than the number of treponemas. However, the serous antibody rate is certainly proportional to the number of treponemas present in the organism.

In the face of clinically equivocal or typical manifestations of recent or tardy syphilis, serology again represents an essential element of diagnosis.

Cephalorachidian liquid lends itself to the same investigations as does serum. Its study furnishes appreciable elements for diagnosis of neurosyphilis.

Serology of syphilis has the benefit of nearly 60 years of experience. At the risk of offending some, it is indispensable to do some trimming in order to draw a relatively clear picture of the resources which the clinician has a right to expect in order to establish his diagnosis.

The serological reactions most widely used in France are classified in the following table:

<u>Reaction to Non-Treponemic Antigens</u>	<u>Reactions to Treponemic Antigens</u>
Fixation of complement	Non-pathogenic treponemas:
Shortincubation at 37°: Dextrache	Reiter: fixation of complement
Debains	
Long incubation at + 4°: Kolmer	Pathogenic treponemas:
Agglutination-flocculation: Kline	Nelson Test: TPI
VDRL (Venereal Disease Research Laboratory)	
(Kahn)	
Opacification-Clarification: (Vernes)	Immunofluorescence: FTA
(Meinicke)	

Reactions to Non-Treponemic Antigens

Fixation of Complements (C'):

In 1901, Bordet and Gengou described the fixation of C' (guinea-pig serum) by means of immune antigen-antibody complexes and revealed this fixation by a hemolytic system: red corpuscles from sheep sensitized by an antishoop antibody (hemolysin or amboceptor). The reaction is effected in two phases:

I

Antigen + serum under examination + C'

II

+ GRS

The response from this reaction is interpreted in function of the following data:

I

Antigen + C' = non-fixation

Antibody + C' = non-fixation

Antigen + antibody + C' = fixation

Hemolysis = absence of antibody

Non-hemolysis = presence of antibody

II

+ GRS = hemolysis

+ GRS = hemolysis

+ GRS = absence of hemolysis

The presence of the specific serous antibody evidences the presence of the homologous antigen.

In 1905, Schaudinn and Hoffman discovered the pale agent *treponema* of syphilis.

In 1906, Wassermann, Neisser, and Bruck applied the Bordet reaction to serodiagnosis of syphilis by using as an antigen an aqueous liver extract from syphilitic stillborn infants (liver very rich in *treponemas*, replacing the unobtainable culture of this microorganism).

With such an antigen, Wassermann observed the fixation of C' by the serums from syphilitics and non-fixation by the serums from normal subjects; thus he thought he had revealed an anti-*treponemic* antibody, which later was to be called "Wassermann's reagin."

In 1907, Levaditi and Marie showed that aqueous or alcoholic liver extracts from non-syphilitic stillborn infants behave in the Wassermann test like liver extracts from syphilitic stillborn infants.

Hence it is not the *treponema* which plays the role of antigen in the Wassermann test, but a "substance" present in extracts from organs of human or animal origin. Landsteiner made it clear in 1908 that the Wassermann antigen is of a lipidic nature and that one can replace an extract from an organ with an alcoholic extract from its lipids. Thenceforth it has been considered that the Wassermann test does not detect a true anti-*treponemic* antibody. In spite of the obscurity clouding the exact mechanism of this reaction revealing a "reagin" (which no one makes bold to call an antibody any longer), this method was widely used and provided useable results for serodiagnosis of syphilis.

Numerous variants of Wassermann's original technique were later described. Among the best known in France, we mention Hecht's reaction (1909) to fresh serum (conveying the C, the hemolytic antibody and possibly the reagin); the Calmette-Messel reaction (1911) with several doses of C', furnishing a relative quantitative notion; the Demanhe and Debains reactions (1933-1934); and the Kolmer reaction (1935-1940), very carefully balanced and the prototype of long-incubation cold reactions with low concentration of C' and red corpuscles.

The antigenic reactives used in these reactions were of empirical preparation, variable in quality from one batch to another. The result was frequent discordance in the responses furnished by these techniques. In the same way, the delicate adjustment of the hemolytic system proved to be a cause of error.

The Opacification-Clarification-Flocculation Reactions:

Thus, the serologists tried to discover the complex formed by the Wassermann antigen and the syphilitic serum without using fixation of C'. Thus there came into being methods making it possible to detect the formation of insoluble precipitates by clarification, opacification, and agglutination. In France in 1917-1918, A. Vernes prepared an alcoholic extract of horse's heart which, placed in contact with serums from syphilitics, causes an opacification whose intensity is read with precision by the photometer. In Germany, Meinicke, Sachs Georgy used other detectors. Kahn's agglutination-flocculation technique (1926), with antigenic preparation sensitized by cholesterol, was very widely applied. On the eve of WW II, the specificity and sensitivity of a multitude of techniques were improved, but the basic mechanism of syphilis serology was still unknown. Fixative, opacifying, or flocculant properties of syphilitics' serum were referred to without considering the Wassermann reagin as a true antibody, still less an anti-treponemic antibody. Some thought that the reagin was a "substance" produced in response to an abnormal lipidic tissue component developed in the course of treponemic infection (autoantibody).

In the United States in 1941, Mary Pan_orn [illegible], analysing alcoholic extracts from beef heart, discovered the specifically active fraction in Wassermann's antigen. This fraction, isolated in the pure state, is a complex phospholipid responding to a chemically defined formula; it is cardiolipid (or cardiolipin).

It has been demonstrated that cardiolipid is a hapten -- i.e., a substance capable of reacting with an antibody in vitro, but incapable of bringing about the formation of this antibody by injection in the animal. When this Wassermann hapten is coupled experimentally with proteins (human serum, pig serum) and then inoculated into a rabbit, the rabbit produces an antibody behaving like the Wassermann

reagin present in the serum of syphilitics.

It has also been demonstrated that cardiolipid is present in a large number of living organisms: it is found in all the animals, in certain microorganisms in the vegetables. Despite this very wide distribution of cardiolipid in the living world, it is established that the corresponding antibody, Wassermann's reagin, is found practically only (with very rare exceptions) in the serum of individuals afflicted with treponematoses (syphilis, yaws, etc.).

Cardiolipid has been found in the body of the pale treponema, and there are strong indications that only the cardiolipid present in pathogenic treponemas (probably linked to specific proteins of these treponemas) is naturally active as a complete antigen in vivo and gives rise to the reagin. Inoculation into an animal of non-pathogenic treponemas (Reiter) containing cardiolipid does not cause the formation of reagin.

Inoculation into an animal of living pathogenic treponemas containing cardiolipid brings about the formation of reagin. However, inoculation of dead pathogenic treponemas, which causes only very slight tissue lesions in the host, also brings about the formation of reagin.

Thus, cardiolipid of pathogenic treponemas is considered by many immunologists as the true antigen causing the formation of reagin in syphilitics, and cardiolipid from other sources, as the hapten supporting the specificity of the reactions to non-treponemic antigens.

The reagin has been obtained in the pure state from serums from syphilitics subjected to different immuno-chemical treatments. It has been identified as an antibody globulin whose electrophoretic character varies during the course of syphilitic infection. It is first of all a 7 S gamma globulin, then a 19 S globulin; it is reported to be found even in the alpha globulins in the serums derived from tardy syphilis. What is beginning to be known about the heterogeneity of the antibodies, mainly thanks to Porter's work, opens up the possibility of distinguishing in the future between the reagin which evidences recent syphilis from the corresponding reagin for tardy syphilis. In other words, it may become possible to differentiate the antibodies corresponding to a non-evolutive syphilis from those linked to an evolutive syphilis.

A number of absorption experiments have clearly demonstrated that Wassermann's reagin is distinct from the immobilising antibody and from the group antiprotein.

In summary, Wassermann's reagin is an antilipidic antibody

forming complex immunes with cardiolipid. Cardiolipid is present in all the non-treponemic antigens in variable proportions, no matter what their mode of preparation.

Complement-Fixation and Flocculation Reactions Using Cardiolipidic Antigen (So-Called "Classical" Serology):

Cardiolipid (whether resulting from an empirical preparation or better, from a standardized method), employed in isolation in these reactions, is only very slightly antigenic: its serological properties depend mainly on the form in which it is presented.

It has long been recognized that certain substances present in variable quantities in organ extracts, such as cholesterol and lecithin, can act, by non-specific physical-chemical phenomena, as adjuvants or additives, considerably increasing the antigenic power of certain preparations. (We recall, for example, Demanche's antigens: "normal" antigen, not very sensitive; "cholesterolated" antigen of higher sensitivity.)

The antigens best controlled at present all contain cardiolipid, lecithin, and cholesterol. These three products are soluble in alcohol.

Cholesterol, which is insoluble in water, precipitates and forms crystals. Lecithin, emulsified in water, places itself on the surface of the cholesterol crystals and acts as a stabilizer.

Cardiolipid, which has a strong affinity for water, arranges itself on the surface and sticks to the lecithin.

For example, in the particles of Kline's antigen (flocculation), the cardiolipid is found at the very surface, presented to the antibody molecules. These particles, scattered, are big enough to be visible under the microscope at low magnification. But it is important to adhere to the optimum ratios between cardiolipid-lecithin and cholesterol. The particles of Kolmer's antigen are smaller than those of Kline because of the different ratios among cardiolipid, lecithin, and cholesterol.

Mechanism of Kolmer's C' Fixation Reaction with Cardiolipidic Antigen:

In a first phase, the serum, previously decomplexed by heating for 30 minutes at 56°, is added to the antigen and to the complement introduced in a limit dosage determined by preliminary testing. The mixture is incubated for 16 hours at + 4°.

-- If the serum contains antibodies, there is first a

cardiolipid-reagin union, and then fixation of C'.

-- If the serum does not contain antibody, antigen and C' remain free.

The long incubation favors the antigen-antibody union; in order not to deteriorate the complement, this incubation is done at + 4°.

After reheating at 37°, the red corpuscles sensitized by hemolysin are added. The reading is taken with the naked eye when there is total hemolysis in the control samples. The response is described in accordance with the following code:

total hemolysis = - = negative = absence of antibody
partial or nil hemolysis = ±, +, ++, to +++ = positive = presence of antibody

Each serum must be tested simultaneously in a control tube, in the absence of antigen. In effect, a certain number of serums may be anticomplementary: they impede the action of hemolytic C'.

In practice, it is mainly a case of serums highly contaminated by bacteria; more rarely, it is aggregates of gamma globulins formed on heating which fix C'; finally, certain serums may collect Ca⁺⁺ and Mg⁺⁺ ions -- ions which are necessary for the action of hemolytic C'. An answer: hence, anticomplementary serum does not in any case make it possible to prejudge the presence or absence of antibody.

Kolmer's technique, widely used at present, has a high sensitivity and good specificity. Even more precise methods are based on definition of the C' concentration by 50% hemolysis. Before adopting them, it should be determined whether they are compatible with the conditions of precision for routine work.

A disadvantage of the Kolmer technique has to do with the duration of this reaction -- about 18 hours. Thus, in practice less sensitive and less specific techniques are still used: the Demanche, and Debains techniques, whose short incubation -- one hour at 37° -- makes it possible to obtain rapid and on the whole correct results.

Recht's technique and its derivatives, using the serum under examination as the complement source, should be completely abandoned because of the imprecision resulting from the variations in C' content and hemolysin in different human serums.

Mechanism of Agglutination-Flocculation Reactions (Kline, VDRL):

The serous antibody molecules fix themselves on the antigenic particles. Agitation encourages antigen-antibody union, then the formation of a network of agglutinates, antibody molecules, bivalent, joining two antigenic particles to each other. Since the antigenic particles comprise a large number of available valences, many particles can be joined to each other by the antibody molecules. The more antibodies there are in the serum, the bigger are the agglutinates. In the presence of normal serum, the fine antigenic particles remain scattered. These reactions are carried out on foil and the results of microscope reading under low magnification are recorded in accordance with the same code as for the C' fixations.

On rare occasions, serums very rich in antibodies can produce a zone phenomenon under the standard reaction conditions: the excess of antibodies prevents the formation of the network and the agglutinates. This zone phenomenon can be detected by a quantitative reaction done on a series of serum dilutions: the reaction, which is negative with the highest serum concentration, becomes positive with lower concentrations and becomes negative again at very high dilutions of serum.

This zone phenomenon is observed more frequently with the agglutination-flocculation reactions than with the C' fixation reactions. In fact, it is quite evident that these different methods, if carefully balanced in function of the serums' usual antibody contents, can be inaccurate in some exceptional cases. All these techniques require scrupulous adherence to the correct proportions of reactives, precise conditions of time, temperature, pH agitation, etc.

This is why perfectly standardized techniques, of simple execution, should be adopted: the Kline or VDRL reactions answer better to these necessities than the classical reactions of Kahn (empirical antigen, complicated original technique), Meinicke, etc.

Qualitative Reactions and Quantitative Reactions:

The object of qualitative reaction is to determine the presence or absence of antibodies in the serums introduced at a single, maximum concentration (pure serum). The response furnished can be:

negative: -
doubtful: ±
positive: +, ++, or +++.

The "positive" response thus expressed reflects a very partial

quantitative notion. Of course, a serum giving a + reaction contains fewer antibodies than a serum giving +++.

However, the extent of serum dilution at which the antibody is still detectable should be made clear, by a quantitative measurement -- i.e., examination of a series of dilutions of positive film.

A positive serum +++ upon qualitative examination may be negative at a dilution of 1:2. Another serum may still be positive at a dilution of 1:64, then negative at 1:128. Thus, by successive doubling of serum dilution, the highest serum dilution giving a clearly positive reaction will be defined: the serum concentration is then expressed by the denominator of the dilution fraction. For example:

<u>Dilutions</u>	<u>1:1</u>	<u>1:2</u>	<u>1:4</u>	<u>1:8</u>	<u>1:16</u>	<u>1:32</u>	<u>1:64</u>	<u>Concentration or Units</u>
Serum No. 1	+++	-	-	-	-	-	-	1
Serum No. 2	+++	+++	+++	+++	±	-	-	8
Serum No. 3	±	+++	+++	+++	+++	++	-	32

Serum No. 3 is an example of a zone phenomenon detected by quantitative reaction.

In practice, it is preferable to adopt a simple, easy to read technique, such as Kline's reaction, as a quantitative method.

The Vernes method measures the intensity of opacification on a single dose of serum and is expressed in photometric degrees. While the quantitative reactions are proposed mainly for establishing a curve reflecting the evolution of the antibody rate in time, after treatment, these reactions are also of interest on the diagnostic level.

Actually, it is exceptional -- at least in continental France -- for a serum with a concentration of four or more Kline units to come from a non-syphilitic subject. On the other hand, it does not seem that the size of a serum's concentration makes it possible to prejudge with certainty the evolutive potential of the syphilis. There exist tabs in evolution, syphilitic aortites accompanied by low reagin rates. Inversely, some subjects with high reagin rates in their serum go many years without presenting any clinical sign of syphilis.

Sensitivity and Specificity of C' Fixation and Flocculation Reactions:

The reactions effected with non-treponemic, cardiolipid-base antigens associate -- as do many immunological methods -- non-specific physical-chemical phenomena and specific phenomena. These methods are

balanced so that under the standard conditions, their specificity and sensitivity are as high as possible. This state of equilibrium, this compromise between specificity and sensitivity, leads one to expect inaccuracies inherent in these methods themselves, not to mention technical failings. The C' fixation reactions -- especially Kolmer's technique -- are more sensitive than the flocculation reactions. (A serum giving 64 units in Kolmer's technique will not give more than 16 easily in Kline's.)

However, the C' fixation reactions are technically delicate, and in practice are more subject to technical shortcomings than the flocculation reactions.

The specificity of the two types of methods (Kolmer, Kline) is entirely comparable, and on the whole, excellent. It has not yet been proven whether the fractions of antibody globulins detected by these two types of reaction are identical or different. Do discordances difficult to explain solely by the differences in sensitivities of these methods express the fact that fixation of C' detects two globulinic fractions and flocculation only one?

The Problem of False Reactions:

The serums of certain subjects who present no antecedent or clinical sign of syphilis and who are apparently healthy or afflicted with maladies having no relation with the treponematoses react with Wassermann's antigen. With the serums of these subjects, there are strong indications that specificity is lacking in the lipidic reactions, and according to a disputable terminology these patients belong to a composite group of "falsely positive biological reactors." The causes suggested to explain these false reactions, long recognized, are many, transient or nearly permanent. The notion of false reaction should be reserved for those subjects examined clinically in a very thorough manner, and after patently positive responses are obtained to several lipidic reactions made at close intervals on several samples of serum from the same subject. Furthermore, at the present time this notion of false positivity can be adopted only if the Nelson test of immobilization of treponemas is negative. If such criteria are adhered to, false reactions are extremely rare in continental France: on the order of one out of 25,000. This extremely low proportion of false reactions obtained in personal experiences is in flagrant contrast with the data furnished by certain authors. In the United States, Moore has asserted that about 40% of white subjects, of a high social level, could present positive lipid reactions without syphilis: the discovery of these false positivities is causing clinical and biological studies sometimes disclosing collagenoses which are still inapparent -- disseminated lupus erythematosus in particular.

There are few bacterial, viral, or parasitic infections or disorders of serous globulins linked with tumoral or immuno-pathological processes which have not been suggested as playing a part in false reactions. Studies made in an African milieu have showed us that, contrary to the conventional data, leprosy is not responsible for false reactions: in the vast majority of cases, positive lipidic reactions in lepers are accompanied by a positive Nelson test, and the frequency -- often high -- of positive serologies in these afflicted persons is found also in the populations from which they come.

In Paris, the rare cases of false reactions which we have observed concern: cases of disseminated lupus erythematosus, leucosis, Hodgkin's disease, hepatitis, myeloma, severe lipoidic nephrosis, heart disease.

In the final analysis, the existence of these false reactions should not be ignored or their frequency overestimated. Reactions to treponemic antigens play an important part in their diagnosis.

Micromethods and Ultrarapid Methods:

In various circumstances, it is not possible to collect a quantity of blood furnishing the volume of serum required by the standard techniques. This is often the case with babies, in cases of tracking syphilis down rapidly in sizeable population masses in movement, or in regions not equipped with well-provided serology laboratories. For a long time, many techniques have been adapted to the availability of small quantities of serum, sometimes collected on blotting paper by simple puncture of a finger. Chediak's technique, for example, which requires special equipment, has been widely used in Central America and in Germany. Demanche adapted his personal method for C' fixation to examination of dried blood.

At present, in fact, the methods of flocculation on foil -- Kline, VDRL -- are micromethods requiring a very small volume of serum (0.05 ml). However, in order to cope with different detection circumstances involving many samples, with little equipment, several technical methods have been developed in recent years by the Venereal Disease Research Laboratory group. Thus, the RPR (Rapid Plasma Reagin) test and the USR (Unheated Serum Reagin) test are simplified variants of the VDRL test, in which the antigen is treated by choline chlorhydrate and a chelator (tetraacetic diamino ethylene acid). Likewise, the Plasmacrit Test is especially economical in cases where microhematocrits are done routinely (personnel of certain industries, etc.). Finally, the Rapid Plasma Reagin Card Test done on blood collected by capillary tube and finger puncture requires neither centrifuge nor microscope. It is obvious that all the subjects presenting positive reactions in the course of detection programs should be reexamined carefully by means of standard

techniques or reference methods with treponemic antigens.

Reactions to Treponemic Antigens

Non-Pathogenic Treponemas:

There are several strains of non-pathogenic treponemas easily cultivable in anaerobiosis. One of the best known is Reiter's strain, isolated in 1922 from lesions of recent syphilis. This saprophytic treponema must have coexisted on the lesions with *T. pallidum*: the latter was lost during attempts at growing it, and only the commensal has been able to be maintained.

In Germany in 1929, Gaetgens used suspensions of Reiter treponema as an antigen in a C' fixation reaction; the results of these reactions were evaluated very differently. In 1944, d'Alessandro and his collaborators in Italy studied the antigenic structure of the Reiter treponema and described three principal fractions:

- a proteic, thermolabile antigen common to *T. pallidum* and *T. Reiter*, active in C' fixation;
- a lipidic antigen, analogous to cardiolipid;
- a polyosidic, thermostable antigen.

By treating the suspensions of Reiter treponemas by various processes, it was possible to prepare antigens consisting mainly of the group protein. The soluble proteic extract of Reiter treponema thus used in C' fixation detects an anti-group protein antibody quite distinct from the Wassermann reagin. These observations by the Italian authors were amply confirmed in France, then in the United States, and the C' fixation reactions with Reiter treponema have entered into current practice. These reactions prove to have a high sensitivity and specificity higher than those of Kolmer cardiolipid. It is very convenient to use the Reiter antigens in the Kolmer reaction conjointly with cardiolipid. However, because of the delicate nature of the C' fixation techniques revealed by hemolysis and wide variations in the quality of the Reiter antigens available on the market, such reactions cannot be considered as reference methods. Certain Reiter antigens possess an anticomplementary power producing hypersensitive and not very specific reactions. It is also important to select the antigens very carefully in order to benefit from the anti-group protein research. The kinetics of this antibody is very comparable to that of the Wassermann reagin.

Pathogenic Treponemas:

Strains of pathogenic pale treponema of human origin, non-cultivable, have been adapted and maintained on rabbits. Thus, the Nichols strain was isolated in 1912 by Major H.J. Nichols of the American Army from the LCR of a patient having a relapse of nervous syphilis.

This strain, inoculated in rabbits, has returned regularly since that time and has kept all its virulence for man, as is evidenced by a number of accidental contaminations in the laboratory and inoculations into human volunteers.

Studies undertaken in 1947 by R.A. Nelson on the culture of pathogenic treponemas led to the development of a medium in which, under an atmosphere of N_2 and CO_2 , these microorganisms can be made to survive for at least 48 hours at 35° . Furthermore, by accelerating the rate of passage to the rabbit testicle, Nelson obtained in these animals acute orchitis very rich in treponemas after eight to ten days of incubation. Suspensions of living treponemas extracted from rabbit testicles and rid of tissue debris by centrifugation thus became available. In 1949, Nelson and Mayer developed the treponema-immobilization test (TPI -- *Treponema pallidum* immobilization).

This was the first reaction using a pathogenic treponemic antigen, regularly reproducible in vitro. The living treponemas brought into contact with syphilitic serum and C' are specifically immobilized, whereas they remain mobile when the serum comes from a non-syphilitic subject. Thus the TPI is a true C' fixation reaction observable directly under the microscope against a dark ground.

The originality of this method concerns the nature of the antigen used (living pathogenic treponema); the direct observation of the conjugate action of an immobilizing antibody and C' on the treponema; the use of a large excess of C' (thus eliminating all variation of sensitivity due to the dosage of C' in reaction -- and this represents a very important difference from the hemolysis reactions); and finally, the long incubation required: 18 to 24 hours at 35° .

Resume of the Technique;

Maintenance of the treponema strain by intratesticular inoculation of six to eight rabbits per week with suspensions very rich in treponemas. Observation by touch of development of rapid acute orchitis, in six to eight days after inoculation.

In order to obtain the suspension of treponemas necessary for

the TPI:

- kill the rabbit;
- collect the testicles sterily and cut them into slices;
- put them in extraction for a few minutes in the Nelson medium;
- centrifugate in order to obtain a suspension rid of tissue debris;
- immediately distribute the suspension into tubes containing for each serum:

	Test tube	Control tube
TP	0.35	0.35
Decomplemented serum to be examined	0.05	0.05
Fresh serum from guinea pigs	0.15	--
Guinea pig serum heated 30 min. at 56°	--	0.15

Place tubes in a desiccator under an atmosphere of 95° N₂ and 5% CO₂. Incubate 22 hours at 35°.

Reading: take a drop from each tube, mount between foil and glass cover and examine under microscope against dark ground (objective 40, eyepieces 10-12X). Mentally count 50 treponemas seen while examining the field ten times and press on a corpuscle-counter for each living treponema.

From the number of living treponemas observed in the test tube and in the control tube, establish the specific immobilization percentage in accordance with the formula:

$$\% IS^* = \frac{(\% \text{ Mobile control treponemas} -- \% \text{ Mobile test treponemas} \times 100)}{\% \text{ Mobile control treponemas}}$$

* [immobilisation spécifique; specific immobilization]

Nelson proposed the following criteria of interpretation, which are still fully valid:

from 0% to 20% - absence of antibody - normal serum
from 20% to 50% - very low rate of antibodies present - serum suspect
from 50% to 100% - presence of antibody - syphilitic serum

Thus, only the immobilizations equal to or higher than 50% present a real diagnostic value.

Thanks to the control tube, any possible treponemicidic power of

the serum, not linked to the presence of antibody, can be detected. Serums loaded with penicillin kill the treponemas in the control tube. This toxicity can generally be removed by neutralizing the penicillin by means of penicillinase. Toxicity due to CyHg cannot be removed. It is improbable that the other antibiotics in current use are responsible for toxicity of serums. On the other hand, strong bacterial contaminations can disturb the reaction and render the result uninterpretable. In this regard it must be remembered that blood samples should be collected sterily from fasting subjects, if possible not under treatment by antibiotics. The blood should be collected in glass receptacles (certain plastic tubes release toxic substances), capable of being sealed hermetically, without anticoagulant (citrate impedes the action of the complement).

Metzger recently demonstrated that addition of lysozyme to the suspension of treponemas makes it possible to shorten the incubation time. The lysozyme is reported to "scour" the treponemas without killing them and accelerate the action of the immobilizing antibody and the complement. It may be asked whether an enzymatic action is indeed involved, for the necessary lysozyme concentration is relatively high. The lysozyme would increase the sensitivity of the TPI. However, this gain in sensitivity may prove harmful to the extent to which a new variable is introduced into a method one of whose essential qualities is that it was balanced admirably by Nelson with only the reactives strictly necessary. In any case, the addition of lysozyme does not solve all the problems raised by maintenance of the strain of treponemas and keeping them in perfect survival.

Since 1950, broad and indispensable clinical experience has shown that the TPI keeps the promises initially advanced by the immunologists. Like all the reactions of neutralization of a virulent antigen by a homologous antibody in the presence of C', the immobilization test has proven to have a very high sensitivity (detection of very low quantities of antibody) and a very high specificity (detection of antibodies directed solely against the pathogenic treponemas).

While the delicacy of the TPI technique limits its use, it imposes on the users a strictness of execution which gives the method its true character as a reference reaction to be used for solving difficult problems (discovery of latent syphilis, tardy syphilis of uncertain diagnosis, false reactions). These indicated uses, theoretically limited, are in fact broadened, for it is often the task of the TPI to throw light on the shortcomings of non-treponemic antigen reaction carried out incorrectly.

in cases of primary syphilitic infections, the immobilizing

antibody appears only at the end of the primary period (an average of 15 days after the Wassermann reagin). From this fact, the Nelson test has no value for early diagnosis of a primary syphilitic infection. On the other hand, in the case of reinfections, such as Degos and we ourselves have observed, the immobilizing antibody reappears in first place. In syphilitics not treated the immobilizing antibody will persist indefinitely (in contrast with the reagin, which disappears spontaneously in a considerable proportion of persons afflicted). While a Nelson test found negative cannot be considered a formal proof of healing, on the other hand the absence of antibody, on repeated examination at intervals of several months or years, is a strong argument in favor of definitive sterilization of the treponemic infection. Does persistence of the immobilizing antibody confer a certain immunity against reinfection? A first experiment carried out by Nelson and Turner in rabbits led these authors to consider that this antibody was, at least in part, the basis of immunity and that its presence excluded the possibility of reinfection. A large breach was made in this concept when it was possible to prove -- first in rabbits, then in human volunteers (Sing-Sing experiment) -- that the subjects carrying the immobilizing antibody could present chancres at the point of cutaneous inoculation with Nichols strain treponemas. Thus a syphilitic, treated but still having a positive Nelson test, can be reinfected by a new influx of treponemas.

The reinfection acts as a recall and is accompanied by a very fast and sizeable reascension of the antibody concentration. This leads us to pose the problem of the quantitative Nelson test.

First of all it must be made clear that 100% immobilization produced by a non-diluted serum (qualitative examination) does not make it possible to prejudge the absolute value of the antibody concentration in this serum. The quantitative test is carried out on a series of serum dilutions (under the same technical conditions as the qualitative test).

The serum's concentration of immobilizing antibody is defined by the highest dilution producing 50% specific immobilization; it is expressed by the logarithm of the inverse of this dilution. The quantitative test makes possible an evaluation, at least approximate, of the antibody concentration. It reveals, for example, that of two serums from different subjects giving 100% immobilization on qualitative examination, one can have an actual concentration of 100 (low concentration), and the other, 2,000 (high concentration).

The value of the information furnished by evolution of the immobilizing antibody concentration after treatment is evaluated diversely. We need not discuss this problem here, but we should emphasize that the reproducibility of the quantitative test is far from

being very satisfactory under routine working conditions. Thus, one should take account only of very large variations in concentration (passage from 100 to 1,000, from 2,000 to 200). On the level of "rough" diagnosis, the quantitative test is of no interest, since the question posed is whether or not there is immobilizing antibody. Whether there is a little or a lot, the fundamental datum is the same: presence of immobilizing antibody signifies syphilis (present or past). Certain authors want to interpret the presence of antibody in a high concentration as evidencing the existence of a syphilis in evolution. Actually, there are tardy syphiles which are symptomatic, evolutive, and accompanied by a very low immobilizing antibody rate; and inversely, certain subjects show high antibody rates for ten years without any syphilitic lesion presented clinically.

In summary, the Nelson test has a very great value for establishing diagnosis of latent and tardy syphiles as well as for exclusion of false reactions. It can legitimately be used as a reference reaction.

Accelerated breeding of the Nelson strain of pathogenic treponemas in the services which do the Nelson test has placed at the disposal of the experimenters suspensions of these microorganisms, living or dead. This source of treponemas has made possible the search for other specific antibodies detectable by less complex techniques than the immobilization test.

Thus it is that in 1952 Nelson, studying phagocytosis of treponemas by the leucocytes, observed the phenomenon of immunity-adherence of treponemas (sensitized by an antibody in the presence of C') to human red corpuscles (TPIA). This attachment of living or dead treponemas to the erythrocytes was detected by counting the treponemas in the supernatant after centrifugation or as we have shown with Nelson, by hemagglutination. Using batches of suspensions of treponemas and C', very carefully selected, we were able to show that immunity-adherence is a specific and sensitive process for detecting anti-treponemic antibodies. Unfortunately, this method has proven unuseable in continuous work because of a high frequency of non-specific reactions encountered with many batches of treponemas and C'.

Likewise, in 1953 Charlotte McLeod and Magnuson, at the Venereal Disease Experimental Laboratory, Hardy and Hollander in Johns Hopkins University, and Cain in Canada described techniques for agglutination of treponemas by serums from syphilitics (TPA). These techniques, attractive in their simplicity, proved to be unuseable in everyday practice because of the spontaneous agglutination of many suspensions of treponemas or agglutination under the influence of serums from non-syphilitic subjects.

In 1955, Magnuson and Portnoy described a technique for fixation of C' (Kolmer type) carried out with an antigen composed of a suspension of pale treponemas treated with sodium desoxycholate (TPCF).

This method, which has very high sensitivity, perhaps greater than that of the Nelson test, has not been widely used in practice because of the anticomplementary power of the antigen and the high cost of its preparation. Furthermore, like all C' fixations detected by hemolysis, this technique is very sensitive in measuring the concentration of C'. Thus, apart from the Nelson test, these various reactions to treponemic antigens, to which is added a reproduction of Pfeiffer's classical phenomenon in the guinea pig, are not indispensable in the current practice of serodiagnosis of syphilis.

On the other hand, since 1957, under the impetus of Harris, Deacon, and collaborators in the United States, the immunofluorescence method, in the indirect process form, has been applied on a constantly larger scale to research on treponemic antibodies. This fine method, introduced in France by Borel and Durel in 1959, derives from the work of Coons, who in 1941 described a process making it possible to render the antigen-antibody complexes visible by coupling a fluorescent colorant to antibody globulins, without denaturation of their immunological properties. The marked globulins fix themselves on the homologous antigen, making it fluorescent and observable under ultraviolet light. In practice, it was impossible to use this direct process, since marking of the serums to be examined by the fluorescent colorant is a long and delicate operation.

On the other hand, the indirect immunofluorescence process appears especially attractive for detecting the antitreponemic serum antibodies. This process involves two phases:

-- first of all, application of the unmarked antibody (serum under examination) on the antigen;

-- then, visualization of the antigen-antibody union by application of a fluorescent indicator. This indicator is a serum of human antigammaglobulins, conjugated to fluorescein isothiocyanate. Thus, a single fluorescent compound is sufficient for examining a large number of samples of human serums. When the necessary reactives (treponemas and conjugate) and an ultraviolet light source are available, this method is very attractive in its rapidity and simplicity. Moreover, the very characteristic morphology of the pale treponemas makes it relatively easy to interpret the images observed under the microscope against a dark ground.

Thus, the FTA (Fluorescent Treponemic Antibodies) test consists

in fixing on foil a suspension of pathogenic treponemas (Nichols strain bred in rabbits), and then depositing on this antigen a drop of the serum to be examined. After a short incubation in a moist chamber, the preparation is washed and then recovered by a drop of marked anti-globulin serum. After washing, the preparation is mounted with a drop of glycerine and a glass cover. The reading is made immediately under a microscope equipped with adequate filters. With normal serum, the treponemas are not fluorescent; serums loaded with antibodies, retaining the antiglobulin, make the yellow-green treponemas -- of more or less intense brilliance -- appear.

The reading is noted in the following form:

--or+ = vaguely visible	= negative
1+ = weakly fluorescent	= negative
2+ = moderately fluorescent	= positive
3+ = strongly fluorescent	= positive
4+ = very strongly fluorescent	= positive

It is immediately apparent that this notion of the intensity of the fluorescence observed is open to dispute, for the difference between 1+ (negative) and 2+ (positive) is far from always being decisive with serums containing a low concentration of antibody.

If each of the principal elements of the reaction is reconsidered in isolation, it has to be noted that no standardization of the re-actives exists and that many technical details vary in the hands of different users. Each author regulates the sensitivity and specificity of the FTA test in function of reference elements: clinical data, cardiolipid reaction, Nelson test. It is clear that the sensitivity and specificity of the FTA test are functions of many factors, of which the principal ones are:

a. The Quality of the Antigen -- Not all the suspensions of treponemas, Nichols strain, obtained from rabbits, possess an identical reactivity. The antigenic characteristics of the treponemas can be more or less modified by the reactions of the host, by the treatments they have been subjected to in preparation and conservation, and by the mode of spreading and fixation on foil. It is proven that the treponemas must undergo surface modifications in order to hold the antibody and the conjugate, since in the absence of desiccation on foil, in liquid medium in the tube, the treponemas do not retain the fluorescent colorants.

b. Dilution of the Serum To Be Examined -- The initial dilution of the serum is a point of prime importance since it is recognized by all that serum from a number of non-syphilitic subjects introduced into

the reaction in a pure or only slightly diluted state causes a distinct fluorescence of the treponemas. In order to eliminate this so-called "non-specific" fluorescence, Deacon has proposed initial dilution of 1:200 for all serums. Is there a critical dilution rate, valid for all serums, on the basis of which any observed fluorescence can be considered specific? This problem, which is without importance for serums very rich in antibodies, is a fundamental one for low-antibody serums, such as are found in the initial and tardy phases of syphilitic infection.

8: The Quality of the Marked Antiglobulin Serum == Depending on the product and the author, this serum is used pure or diluted 1:40, 1:200, etc. The preparation of these serums has remained very empirical up to the present. Will attempts at purification of these serums, selection of active fractions by passage through Sephadex and chromatography on cellulose DEA column, or couplings, in fixed proportions of molecules, of antibody proteins with molecules of colorant lead to elimination of the so-called "non-specific" colorants? At present, the products obtained are of very variable activity as between one batch and another. This is still one of the major shortcomings of the method.

On the other hand, no experimental study has yet made it possible to define exactly the antigen or antigens and the antibody or antibodies implicated in immunofluorescence.

The evolution of the long-term antibody in the course of untreated syphilis of the rabbit has not yet been established. It is known only that very high antibody rates (20,000) are sometimes observed in humans afflicted with secondary or tardy syphilis. It would seem that the antibody appears very early in the course of natural infection; detection of it could constitute a valuable element for diagnosis of incipient infections. With regard to the evolution of the antibody in cases of untreated syphilis in the symptomatic latent or tardy phases, the present data are contradictory. According to some, the immunofluorescence antibody persists indefinitely, like the immobilizing antibody; in the opinion of others, it disappears spontaneously in a considerable number of untreated afflicted persons (Oslo study on untreated syphilis: Eng, Nielsen, and Wereide).

In the present state of the technique of testing fluorescent treponemic antibodies, and according to the available information on the sensitivity and specificity of this method, it would be entirely premature to assign a definitive place to the FTA test in the arsenal of immunological techniques applicable to the diagnosis of syphilis.

For certain users, the FTA test -- which is a very sensitive and

very specific method, with excellent reproducibility -- is the ideal reaction for application to the diagnosis of syphilis in all its stages, especially at the very beginning of infection. A micromethod has recently been proposed for detection of syphilis in a long series of samples (Vaisman, Hamelin, Guthe). In brief, the FTA test is recommended as a replacement for the cardiolipidic reactions, even for the Nelson test. This idea -- a highly optimistic one, argued mainly by Deacon, Fribourg-Blanc and Niel, Vaisman and Hamelin -- is far from being shared completely by other experimenters, such as Nielsen, Thiviolet, Miller and collaborators, and ourselves.

Indisputable as the value of the immunofluorescence method may be on the plane of research, generalization of its use on the plane of everyday diagnosis of syphilis seems premature to us; until true standardization of the method is realized, the clinicians run the risk of obtaining contradictory information.

By way of conclusion to this study of the treponemic antigen reactions, we hold that it is in this group that the most specific and most sensitive methods are found. It is in this area that the most active experimentation is being carried on -- notably, analysis of the antigenic structure of the pathogenic treponemas. However, this research remains complicated by the impossibility of cultivating the pale treponema in synthetic mediums. The suspensions of treponemas obtained from rabbits remain more or less loaded with products from the host, and these products can interfere with the immunological phenomena.

Significance of Responses From Serological Reactions

The antibodies detected by the different methods which have been described have kinetics more or less disparate in time. The serological methods have different sensitivities and specificities.

Hence it is not surprising that even apart from any technical shortcomings (and unfortunately, such shortcomings are not rare), the responses from the various serological reactions are not always in agreement among themselves.

With the object of simplification, we shall reconsider in summary form the evolution of antibodies in the course of untreated syphilis, taking:

1. the Wassermann reagin, detected both by the C' fixation reactions (Kolmer) and by the flocculation reactions (Kline) carried out with cardiolipidic antigens;

2. the immobilizing antibody detected by the Nelson test (TPI);

3. the group anti-protein detected by the fixation of C' with proteic antigen extracted from the Reiter treponema;

4. the antibody detected by immunofluorescence (FTA).

Recent Syphilis -- Primary-Secondary:

The FTA would seem to appear first, 15 days after the beginning of the chancre. The reagin and anti-group protein appear about three weeks after the beginning of the chancre.

The immobilizing antibody appears at the end of the chancre and at the beginning of the second period (except in the case of reinfection, when it often reappears first).

These antibodies attain the highest concentrations at the end of the secondary period:

Reagin:	64 to 256 units
Reiter:	64 to 256 units
TPI:	1,000 to 3,000
FTA:	3,000 to 30,000 (?)

Latent Syphilis:

Essential phase for serological diagnosis, very valuable in duration, from end of the secondary period to the tardy phase.

Reagin:	generally present in very small concentrations; sometimes absent
Reiter:	generally present in variable concentrations; some- times absent
TPI:	always present in more or less high concentrations
FTA:	generally present; sometimes absent (?)

Asymptomatic Tardy Syphilis:

Simple prolongation of latent syphilis in time. Indefinite duration.

Reagin:	often absent
Reiter:	often absent
TPI:	present
FTA:	?

Symptomatic Tardy Syphilis:

(Gummas, nervous and cardiovascular syphilis)

Reagin: present in low or high concentration; often entirely absent
Reiter: present in low or high concentration; sometimes absent
TPI: generally present in low or high concentration; very rarely absent
FTA: ?

In summary: the reagin appears early, but can disappear quite fast (of diagnostic interest at beginning of syphilis).

The anti-group protein seems to persist a little longer than the reagin.

The immobilizing antibody, late in appearance (without diagnostic interest at the beginning of syphilis), persists almost indefinitely (of interest for tardy diagnosis).

The immunofluorescence antibody appears very early. Its subsequent evolution is uncertain.

Interpretation of Results

The results of serological reactions require first of all to be interpreted with the individual qualities of sensitivity and specificity of the different methods taken into account. In cases in which clinical examination reveals nothing, interpretation of the results will be valid only if at least two samples of serum for the same subject are examined at an interval of several days and give clear and concordant responses.

However, it is not necessary to expect clarification from a multiplicity of reactions, especially if they are intended to detect the same type of antibody. If two cardiolipidic reactions (Kolmer, Kline) raise a diagnostic problem, the solution will generally be furnished by a control reaction -- the Nelson test.

In cases where this is not practical, use should be made of the reaction with Reiter antigens, and perhaps, in the future, immunofluorescence.

To simplify in the extreme, the following eventualities may be

taken:

<u>Reagin</u>	<u>TPI</u>	<u>Interpretation</u>
+	+	syphilis certain, current or past interest of reagin concentration
-	-	healthy subject, or cured syphilitic, or beginning syphilis (chancre)
+	-	beginning syphilis, false biological reaction
-	+	latent or tardy syphilis

On the other hand, if one takes the whole of the serological responses and compares them with the clinical examination, the following eventualities will be seen:

Concordances:

Clinical - serological - : healthy subject or cured syphilitic

Discordances:

1. Clinical + serological - : tardy syphilis (very rare)
2. Clinical - serological + : latent syphilis (frequent)

Data Furnished by Cerebrospinal Liquid (CSF):

All the reactions applicable to serum can be carried out on CSF. However, in certain cases it is necessary to concentrate the CSF proteins, for as a general rule, the antibody rate is less high in this liquid than in the blood. Complete observations making it possible to suspect antibody production localized at the neuraxis are very rare. In the vast majority of cases, the antibodies are present simultaneously in the CSF and in the serum; whence a strong tendency to consider that the antibodies pass from the blood into the CSF through the meningeal barrier. On the diagnostic level, it is very exceptional for the discovery of antibody in the CSF alone to contribute decisive information. In the case of the frequent meningeal reactions accompanying secondary syphilis, the antibodies are present in the serum and the CSF. Because of the high sensitivity of the Nelson test, the immobilizing antibody can be detected in the CSF when the reagin is absent from it (especially in tardy syphilis). Up to the present, we have never observed positive TPI in CSF and negative TPI in the serum. This notion, which is perhaps

not absolute, removes a great deal of the diagnostic value from isolated search for antibody in the LCR. On the other hand, association of a positive TPI in the serum and in the LCR provides an important indication of injury of the nervous system.

Furthermore, LCR lends itself to:

1. Quantitative determination of the albumin rate (normal rate: 0.25 g per 1,000 ml) or the total protein rate (usually higher than 40 mg per 100 ml in neurosyphiles). The Pandy and Nonne tests are of limited value.

2. Counting of cells (normal: less than 2 elements per mm³), which should make possible in particular recognition of the moderate forms of asymptomatic neurosyphilis.

3. The non-specific test of precipitation of colloidal benzoin. In neurosyphiles, the benzoin suspension flocculates in the tubes in which the LCR concentration is high, contrary to what happens with normal LCR. Flocculation is expressed in figures: 0 1 2.

Normal liquid: 0000002200000000

PG liquid: 2222222220000000

This non-specific reaction has lost much of its interest since the use of the Nelson test.

Particular Case of Infants Born to a Syphilitic Mother:

It is generally granted that the treponema traverses the chorial villusities of the placenta only from the fifth month of pregnancy. Hence it is only after the fifth month that the foetus can be infected and begins to produce its own antibodies, although the foetus's and newborn baby's capacity for producing antibodies is judged in different ways and can be influenced by immunological tolerance phenomena.

In practice, two problems concerning congenital syphilis are to be considered:

1. The passive transfer of maternal antibodies to the infant without infection of the latter. The infant, having received only the antibodies from the mother (treated), eliminates them in a few weeks or months after birth. This gradual elimination should be followed by quantitative reactions (Kline, for example). The anti-lipidic antibodies disappear faster than the immobilizing antibody.

2. The foetus has been contaminated more or less late in the

course of pregnancy, or even upon delivery. The newborn may possess antibodies from his mother, his own antibodies, or no antibodies (contamination upon delivery). Therefore it will be useful to compare the antibody rate in the serum of the mother and of the infant; in the latter, the rate will gradually rise in the case of tardy infection, and will subsequently evolve as in the adult.

Allergy in Syphilis:

Since the beginning of this century, the concept of the intervention in syphilis of a hypersensitivity comparable to that of tuberculous persons to tuberculin has been recognized. According to Bordet, this hypersensitivity has the effect of giving to pathological neoformations an importance disproportionate to the number of germs which they contain. Very many attempts at exploration of this allergy by intradermic inoculation of products containing treponemas have been undertaken since 1906. For a long time, the results of intradermoreactions carried out with "luetins" of different origins were subject to discussion, and the specificity of this test was uncertain. In 1941, Degos, using the luetin of Muller and Brandt, prepared from syphilitic orchites of rabbits inoculated with several strains of treponemas, demonstrated the interest of research on hypersensitivity and made it clear that only an allergy was involved.

Since development of the Nelson test (1949), suspensions rich in pathogenic treponemas, relatively free of tissue debris and useable in intradermoreaction after treatment by formalin, have been available. This luetin, composed of formalinated treponemas in suspension in physiological water, has been used widely, notably by Simeray, Gate, Thivolet, and Sohler in Lyon, by Ranque, Tramier and Charpy in Marseille, and Huriez in Lille.

From Simeray's excellent study, it may be held that exploration of cutaneous allergy by intradermic injection of 0.20 ml of luetin is a specific method. The results obtained show the independence of the allergy phenomena of production of serous antibodies. The reaction is negative to the primary-secondary phases of syphilis. It is constantly positive in the forms of late syphilis with cutaneous, osseous and cardiovascular manifestations. Intradermoreaction is not always positive in latent and nervous syphiles.

Thus, on the diagnostic level, exploration of cutaneous allergy is not to be neglected in the tardy forms of syphilis. In practice, one injects 0.10 to 0.20 ml of treponemic suspension into the skin of the deltoid or forearm region. A control is made with an extract from testicles of non-syphilized rabbits. The reaction is read from two to four days after injection. Negative reaction is not accompanied

by a dermic lesion -- at most a slight transitory erythema.

The positive reaction is expressed by the appearance at the point of inoculation, in two or three days, of an erythematous papule of variable diameter, which sometimes can become vesicular or necrotic. The infiltration is maximum on the fourth day, and persists for some 15 days. In function of the diameter and infiltration of the papule, the reading can be noted by: +, ++, +++, or +++.

In summary, the "luo-test" contributes elements useful in diagnosis of tardy syphilis, detecting a hypersensitivity of retarded type which, while it plays no part in immunity, is doubtlessly an aggravating factor of the organic lesions initiated by the treponema.

Influence of Immunity on Diagnosis of Syphilis:

Any subject having contracted syphilis and having received no treatment will have an immunological behavior different from that of a new subject as regards a reinoculation: he will show no primary-secondary accidents; his antibody response will be accelerated; and he may have some gummatous lesions. The state of resistance develops slowly; the effectiveness of immunity is limited and it declines with time. The part played by the antibodies in this relative immunity is very poorly defined in syphilis as in tuberculosis. Since no attempt at vaccination against syphilis has been successful so far, the presence of antibody can only be the result of contracted infection.

Diagnostic Differential as Between Reinfection and Relapse:

The contribution of serology to the solution of this sometimes very difficult problem should be interpreted with prudence, for many factors, practically uncontrollable, can intervene.

Customarily, in cases of reinfections, the reappearance of antibodies is very rapid and the concentrations obtained are high (we recall that the immobilizing antibody may reappear first). In cases of relapse, ascension of the antibody rate can only be very gradual.

Among the factors capable of influencing the production of antibodies, we cite only earliness of treatment and administration of corticoids.

Problem of Other Treponematoses:

The group of treponemas pathogenic for man includes:

Treponema pallidum: causal organism of syphilis

Treponema pertenue: causal agent of yaws
Treponema carateum: causal organism of pinta.

Furthermore, the treponemas responsible for

- bejel in Asia Minor and the eastern Mediterranean
- the endemic syphilis of the Balkans and certain regions of Africa
- dichuwa in Bechuanaland
- siti in Gambia
- njovera in Southern Rhodesia

are considered as identical with *T. pallidum*, and these treponematoses are often grouped under the heading of endemic syphilis or non-venereal syphilis, frequently acquired in childhood. Because of the close antigenic relationships existing among the pathogenic treponemas, the serology of treponematoses represents a typical example of crossed reactions.

It is impossible to distinguish the various treponematoses on the serological level: Wassermann's reagin appears in the course of yaws as well as syphilis. The Nelson test can be made both with *T. pallidum* and with *T. pertenue* to detect immobilizing antibody in syphilitics.

Conclusion

There are remarkable facilities for serological reactions applicable to diagnosis of syphilis.

In practice, from such a rich array -- perhaps too rich --, one should choose a limited number of reactions done with strict technical exactness in the presence of adequate controls.

In the vast majority of cases, the cardiolidipic reactions answer to the requirements of clinical examination and epidemiology. The Nelson test -- of remarkable specificity and used especially for study of litigious cases -- remains the reference reaction.

The position of immunofluorescence cannot be formally defined at present.

After a long period of isolation, the serology of syphilis has come back into the framework of immunology of bacterial maladies. The presence of antibodies is linked to the presence of the treponema.

However, no serological method, no matter how highly perfected,

is completely immune from failure. Only close cooperation between clinician and biologist gives to serodiagnosis of syphilis all its significance and value.

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